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14. ABSTRACT
We have established a GH-deficient, prostate cancer model (Tag/Ghdr/dr rat), indicating that a reduction in GH and/or IGF-I can significantly inhibit prostate carcinogenesis in this model in contrast to GH wild-type controls (Probasin/Tag, Tag/Gh+/+). Tag/Gh+/+, Tag/Ghdr/dr and age-matched non-Tag controls were sacrificed at 10, 25 and 52 weeks of age. While real-time RT PCR and immunohistochemical analysis revealed the significantly increased levels of prostate GHR and the dramatically reduced levels of prostate IGF-1R ($P<0.0001$) in Tag/Gh+/+ during prostate cancer progression, the loss of prostate GHR and the increase of IGF-1R were observed in Tag/Ghdr/dr. However, there was no significant change in either serum or prostate IGF-1 level that can be correlated with prostate cancer progression or the resistance of Tag/Ghdr/dr to prostate carcinogenesis. These findings are consistent with the hypothesis that GH signaling plays a significant role in prostate carcinogenesis and suggest that GH antagonists may be effective agent against prostate cancer.

15. SUBJECT TERMS
Growth hormone, prostate cancer, hormonal prevention

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Introduction

Prostate cancer is the most common and second deadliest form of cancer afflicting American men (1). Androgens are important regulators of prostate proliferation differentiation and apoptosis and androgen antagonism remains the primary treatment for prostate cancer. While initially effective, most patients' tumors re-emerge as androgen independent disease. Clearly, other treatment modalities are urgently needed. Recent clinical and epidemiologic studies illustrate the role of growth hormone (GH) and insulin-like growth factor-I (IGF-I) in normal human prostate development as well as prostate cancer. A major hurdle in the development of novel agents for the treatment of prostate cancer is the lack of appropriate animal models. There currently exist well-characterized rodent models of GH/IGF axis hypofunction. There are also well-established rodent models of prostate carcinogenesis. However, there is no model in which prostate carcinogenesis can be evaluated as a function of the GH/IGF axis. This is the goal of the approved work. The development of such models, Tag/ $Ghr^{-/-}$ mice and Tag/$Gh^{-/-}$ rats, will contribute to our understanding of the role of the GH/IGF axis in prostate carcinogenesis.

Body

Research accomplishments

Objective 2: Develop the first mouse model to test the hypothesis that a normal, functional GH/IGF axis is required for prostate carcinogenesis

This objective has been completed and the results have been published in *Endocrinology.* A reprint of this publication is supplied in the appendix.

Objective 1: Develop the first rat model to test the hypothesis that a normal, functional GH/IGF axis is required for prostate carcinogenesis

This objective has been completed and the results are currently being prepared for submission as two manuscripts to a peer-review journal. The abstracts of these manuscripts follow.

Abstract for manuscript 1:

Previous studies suggested that down regulation of growth hormone (GH) signaling can block prostate intraepithelial neoplasia development in a mouse model of relatively indolent prostate carcinogenesis. In the present investigation, we asked if down regulation of GH signaling could block carcinogenesis in the Probasin/TAg rat, a model of aggressive prostate cancer. The Spontaneous Dwarf rat, which lacks GH due to a mutation (dr) in its GH gene, was crossed with the Probasin/TAg rat, which develops prostate carcinomas at 100% incidence by 15 weeks of age. Progeny were heterozygous for TAg and homozygous for either the wild-type GH gene (TAg/Gh+/+) or the dr mutation (TAg/Ghdr/dr). Prostate tumor incidence was significantly reduced, tumor latency was delayed and tumor burden was significantly reduced in TAg/Ghdr/dr rats relative to TAg/Gh+/+ controls. At 25 weeks of age, loss of GH resulted in a 20% and 80% decrease ($P < 0.05$ and $P < 0.0001$) in the area of microinvasive carcinoma in the dorsal and lateral lobes, respectively. By 52 weeks of age, invasive prostate adenocarcinomas were observed in all TAg/Gh+/+ rats with metastasis, while the majority of TAg/Ghdr/dr did not develop invasive tumors. The absence of GH did not affect expression of the TAg oncogene, prostate AR or serum testosterone titers. These findings suggest that GH signaling plays an important role in progression from latent to malignant prostate cancer driven by the powerful probasin/TAg construct in rats and suggest that GH antagonists may be effective at treating human prostate cancer.

Abstract for manuscript 2:

We recently established a GH-deficient, prostate cancer model (Tag/$Gh^{dr/dr}$ rat), indicating that a reduction in GH and/or IGF-I can significantly inhibit prostate carcinogenesis in this model in contrast to GH wild-type controls (Probasin/Tag, Tag/$Gh^{+/+}$). The purpose of the current study was to further determine if the progression of prostate cancer is associated with changes in sensitivity to GH or IGF-I by determining expression levels of GH receptor (GHR), type-1 IGF receptor (IGF1R) and IGF-I. Tag/$Gh^{+/+}$, Tag/$Gh^{dr/dr}$ and age-matched non-Tag controls ($Gh^{+/+}$and $Gh^{dr/dr}$) were sacrificed at 10, 25 and 52 weeks of age. These cohorts were chosen to represent progressive stages of prostate cancer development in Tag/$Gh^{+/+}$ rats from prostatic intraepithelial neoplasia to macroscopic invasive tumors, which closely mimic that observed in the human disease. While real-time RT PCR and immunohistochemical analysis revealed the significantly increased levels

of prostate GHR and the dramatically reduced levels of prostate IGF-1R (P<0.0001) in Tag/$Gh^{+/+}$ during prostate cancer progression, the loss of prostate GHR and the increase of IGF-1R were observed in Tag/$Gh^{dr/dr}$. However, there is no significant change in either serum or prostate IGF-1 level that can be correlated with prostate cancer progression or the resistance of Tag/$Gh^{dr/dr}$ to prostate carcinogenesis. These findings are consistent with the hypothesis that GH signaling plays a significant role in prostate carcinogenesis and suggest that GH antagonists may be effective agent against prostate cancer.

Characteristics of experimental animals (body weight, prostate weight, and differentiation)

As expected, body weight and length were reduced in TAg/SDR (TAg/ $GH^{dr/dr}$) compared to age-matched TAg/WT (TAg/$GH^{+/+}$) rats at 10 weeks of age (N=10, Figs. 1A), but indistinguishable from SDR. The seminal vesicles, coagulating gland, ventral, lateral and dorsal prostate were all present and reduced in size, but of normal appearance in TAg/SDR rats compared to TAg/WT rats at 10 weeks of age. The average ventral, lateral and dorsal prostate weights were significantly lower (P<0.0001) in TAg/SDR than in TAg/WT (Fig.1A). However, no significant difference in the prostate to body weight ratio was observed between TAg/SDR and TAg/WT rats (Fig.1B), indicating that the reduction in prostate weight is proportionate to the reduction in body weight, consistent with an effect of reduced GH action.

To study the effect of GH signaling on prostate development and differentiation, several biomarkers were evaluated by immunohistochemistry. Markers of prostatic epithelial cell differentiation included p63 for basal cells and cytokeratin 18 (CK18) for the luminal cell subpopulation. Functional differentiation was assessed by immunostaining for probasin and PBP, the major prostatic secretory proteins in dorsal and ventral prostate. In TAg/WT prostates, basal cells (p63+) were intermittently localized along the basement membrane in the central and distal regions of the ventral and dorsolateral lobes (Fig. 2A) and this pattern was not affected by the loss of GH signaling in the TAg/SDR prostates (Fig. 2B). The majority of the prostatic epithelium in both TAg/WT and TAg/SDR stained for CK18, a marker of a differentiated luminal cell (Fig 2, C&D). Furthermore, in both genotypes, probasin and PBP strongly stained in the prostate (Fig2. E-H) indicating that functional differentiation of the epithelial cells was not compromised by the loss of GH.

Prostate carcinogenesis is retarded by deletion of the GH (in situ and metastasis)

Rats were sacrificed at 5, 10 and 25 weeks of age and their prostates were dissected as described in Materials and Methods. The serial sections of prostate lobes were examined histologically for prostate carcinomas (Fig. 3A &B). The incidence and latency of hislogical carcinomas in ventral, lateral and dorsal lobes were shown in Fig. 3C. It indicated that TAg/WT rats have 100% of incidence of carcinoma in ventral lobe even at 5 weeks of age. The latency has been delayed by 10 weeks in lateral and dorsal lobes. The absence of GH axis in the TAg/ SDR delayed latency and decreased the incidence in all three lobes. And at 25 weeks of age, loss of GH resulted in a 79.5% and 19.5% decrease (P <0.0001 and P<0.05) in the area of carcinoma in the lateral and dorsal lobes but was without detectable effect in the ventral lobe (Fig. 3D).

Furthermore, by 1 year of age, 100% of TAg/WT rats, but only 46% of TAg/SDR developed macroscopic tumors in local invasion, with metastasis found in mammary and submaxillary glands (Table. 1).

Expression of TAg

As shown in Fig. 4A, the level of SV40 TAg expression in the lateral lobe was measure by real time RT-PCR. There was no difference ($P < 0.05$) between TAg/WT and TAg/SDR in transcription level, consistent with identical numbers of immunoreactive epithelial cells in these animals (Fig. 4 B&C). Therefore, the lack of the GH in this model does not appear to affect TAg expression in prostate epithelium.

Testosterone levels or androgen receptor expression was unaltered by disruption of GH signaling

Serum testosterone levels were analyzed in groups of adult (16 weeks) male TAg/WT and TAg/SDR. Testosterone levels were not affected by GH status as determined by statistical analysis (Fig. 5A). Furthermore, immunohistochemical analysis of AR demonstrated that there was no difference in AR expression between TAg/SDR and TAg/WT rats in normal or cancerous prostate epithelial cells (Fig. 5B-E).

Prostatic GHR Expression Was Increased during Prostate Cancer Progression but Decreased in Cancer-Resistant Tag/$Gh^{dr/dr}$ Rat.

To examine the specific changes of the GH/IGF axis in the prostate gland during cancer progression, RNA and protein samples from lateral prostates (LP) of Tag/$Gh^{+/+}$, Tag/$Gh^{dr/dr}$ and age-matched non-Tag controls ($Gh^{+/+}$ and $Gh^{dr/dr}$) were analyzed using real-time RT-PCR and immunohistochemistry, respectively. The mRNA expression levels in Tag rats were expressed relative to those in the age-matched non-Tag controls, which can remove the age factor and clearly demonstrate the effect of the prostate lesions on the expression of GH/IGF axis.

As shown in Fig. 6A, the changes in expression of GHR in the prostates of Tag/$Gh^{+/+}$ rats did not occur in 10 weeks when the prostates generally have developed early lesions consistent with PIN and/or well-differentiated adenocarcinomas. However, the significantly increased levels of prostatic GHR were observed in tumors at 25-weeks-of-age when the histological grade of tumors ranges from well differentiated to poorly differentiated adenocarcinomas (4.1 fold), and at 52 weeks of age when the prostates developed into the invasive tumors (up to 17.6 fold). These findings were confirmed by immunohistochemistry (Fig.6B-D), which demonstrate clearly that prostatic GHR is highly up-regulated not in the early stages of cancers (PIN or well differentiated tumor cells), but in the advanced stage of cancer (moderately or poorly differentiated tumor cells).

It was noted that the expression of prostatic GHR in Tag/$Gh^{dr/dr}$, when compared with the age-matched $Gh^{dr/dr}$ control, remained similar (10 weeks and 25 weeks) even lower level (52 weeks) during prostate cancer progression (Fig. 6A & E-G), which may slow the growth of prostate cancer cells and contribute to the refractoriness to the prostate carcinogenesis in Tag/$Gh^{dr/dr}$. Interestingly, GHR levels were significantly increased (1.4 fold) in the invasive prostate tumors of Tag/$Gh^{dr/dr}$ (Fig. 6A & H), which further implicates GHR as an important factor in the development of advanced prostate cancers.

Prostatic IGF-1 Expression Was Unaltered during Prostate Cancer Progression.

As shown in Fig. 7A, the expression of prostate IGF-1 mRNA was significantly reduced in 10-week-old Tag/$Gh^{+/+}$ rats, only 50% of that observed in 10-week-old non-Tag $Gh^{+/+}$ rats. The reduced IGF-1 mRNA level was observed to increase in 25-week-old Tag/$Gh^{+/+}$ rats, but remained at non-Tag levels in 25-week-old Tag/$Gh^{+/+}$ rats as well as in 52-week-old Tag/$Gh^{+/+}$ rats, which developed invasive prostate tumors. The mRNA data were further confirmed by checking the protein level of prostate IGF1 using immunohistochemistry (Fig. 7B-D). There was no noticeable change in IGF-1 expression during the transition from normal prostate epithelial cells to PIN lesions, even in the poorly differentiated cancer cells.

As shown in Fig. 7A & E-G, changes in the expression of prostate IGF-1 (both mRNA and protein levels) did not occur during prostate cancer progression in Tag/$Gh^{dr/dr}$. Even in the invasive prostate tumors (Fig. 7A & H), prostatic IGF-1 remained at levels similar to those in age-matched non-Tag controls. These data suggest that there is no significant change in the prostate IGF-1 level that can be correlated with prostate cancer progression. Furthermore, prostatic IGF-1 may have little effect on the resistance of Tag/$Gh^{dr/dr}$ to prostate carcinogenesis.

Prostatic IGF-1R Expression Was Decreased during Prostate Cancer Progression but Increased in Cancer-Resistant Tag/$Gh^{dr/dr}$ Rat.

As shown in Fig. 8A &B, expression of prostatic IGF-1R in Tag/$Gh^{+/+}$rats did not change in comparison to age-matched non-Tag $Gh^{+/+}$controls during the early stages of prostate cancer in this model (up to 10 weeks of age). Interestingly, however, IGF-1R mRNA levels were significantly reduced (57%) in the prostates of 25-week-old Tag/$Gh^{+/+}$ rats with advanced adenocarcinomas (Fig. 8A & C). The decrease (78%) in the expression of IGF-1R mRNA persisted as the prostate cancer progressed to macroscopic invasive tumors at 52 weeks of age (Fig. 8A & D).

As observed in Tag/$Gh^{+/+}$, the expression of IGF-1R in Tag/$Gh^{dr/dr}$ rats remained unchanged in comparison to age-matched non-Tag $Gh^{dr/dr}$ controls at 10 weeks of age (Fig. 8A & E). However, in contrast to the significantly reduced IGF-1R levels in Tag/$Gh^{+/+}$, the IGF-1R levels were significantly increased (1.7 fold) at 25-week-old Tag/$Gh^{dr/dr}$, and remained high in the majority of 52-week-old Tag/$Gh^{dr/dr}$ rats, which were the non-invasive tumor-bearing animals (Fig. 8A & F-G). Interestingly, the decrease (78%) in the expression of IGF-1R, which was found in the invasive tumors from Tag/$Gh^{+/+}$rats, was also observed in the invasive tumors from Tag/$Gh^{dr/dr}$ rats (Fig. 8A & H). These observations suggest that down-regulation of IGF-1R is necessary for tumor formation. Furthermore, increased IGF-1R expression in 25- and 52-week-old Tag/$Gh^{dr/dr}$ rats contributes to the resistance of Tag/$Gh^{dr/dr}$ to prostate carcinogenesis.

Serum IGF-1 Levels Were Unaltered during Prostate Cancer Progression.

Radioimmunoassays were used to determine whether the changes in the concentration of serum IGF-1 correlated with prostate cancer progression in the Tag/$Gh^{+/+}$ rats or with resistance of Tag/$Gh^{dr/dr}$ to prostate carcinogenesis. As shown in Fig. 9A, the serum IGF-1 concentration in non-Tag $Gh^{+/+}$ rats increased between 10 to 25 weeks of age ($P < 0.05$) but declined by 52 weeks of age ($P < 0.05$). This is similar to the observations in Tag/$Gh^{+/+}$. Importantly, serum IGF-1 in the Tag/$Gh^{+/+}$ rats was comparable to the level in age-matched non-Tag $Gh^{+/+}$ controls during the cancer progression from PIN to invasive tumors. The data indicated that serum IGF-1 did not correlate with either Tag expression or prostate cancer progression in these rat models.

Similarly, it was observed that the concentration of serum IGF-1 reached a maximal level at 25 weeks of age in $Gh^{dr/dr}$ rats, which was 10% of the IGF-1 level found in age-matched $Gh^{+/+}$ (Fig. 9B). Moreover, at any age examined, there was no difference observed between $Gh^{dr/dr}$ and Tag/$Gh^{dr/dr}$ rats, including those that developed into the invasive tumors at 52 weeks of age. Their serum IGF levels were comparable to the low levels in non-Tag controls. The data indicated that serum IGF-1 levels in Tag/$Gh^{dr/dr}$ were unaltered during prostate tumor progression and low levels of serum IGF-1 may not correlate with the resistance of Tag/$Gh^{dr/dr}$ to prostate carcinogenesis.

Key Research Accomplishments

1) Mouse model

- Published manuscript in Endocrinology (see appendix)

2) Rat Model

- Spontaneous Dwarf Rat (SDR) is crossed with Pb/Tag rat to produce the Tag/Gh+/+ and Tag/Gh-/- rat.
- Measured various characteristics of the new rat strain including weigh, prostate weight, and differentiation biomarkers expressed within the prostate
- Monitored prostate development in rats 5, 10, 55 and 52 weeks of age
- Observed a statistically significant decrease in prostate cancer incidence, tumor burden and an increase in tumor latency in rats lacking growth hormone
- Observed that prostatic GHR expression was increased during prostate cancer progression but decreased in cancer-resistant Tag/Ghdr/dr rat.
- Observed that prostatic IGF-1 expression was unaltered during prostate cancer progression
- Prostatic IGF-1R expression was decreased during prostate cancer progression but increased in cancer-resistant Tag/Ghdr/dr rat
- Serum IGF-1 levels were unaltered during prostate cancer progression

Reportable Outcomes

Manuscript (attached in appendix)

Wang, Z., Prins, G.S., Coschigano, K.T., Kopchick, J.J., Green, J.E., Ray, V.H., Hedayat, S., Christov, K.T., Unterman, T.G. and Swanson, S.M. Disruption of growth hormone signaling retards early stages of prostate carcinogenesis in the C3(1)/Tag mouse. Endocrinology, 146: 5188-5196, 2005.

Oral Presentations

Swanson, S.M., Zhang, X., Wang, Z., Coschigano, K.T., Ray, V.H., Shirai, T., Green, J.E., Mehta, R.G., Kopchick, J.J., Prins G.S., and Unterman, T.G. Novel animal models to study the role of growth hormone signaling in carcinogenesis. The Endocrine Society's 87th Annual Meeting, June 4-7, 2005, San Diego, CA.

Wang, Z., Shirai, T., Ray, V.H., Christov, K., Lantvit, D.D., Shah, H., Hedayat, S., Unterman, T.G., Prins G.S. and Swanson, S.M. Disruption of growth hormone signaling substantially retards prostate carcinogenesis in the Tag transgenic rat model. The Endocrine Society's 87th Annual Meeting, June 4-7, 2005, San Diego, CA.

Kopchick, J.J., Pollak, M., and Swanson, S.M. Growth Hormone Receptor (GHR) Antagonists, GHR 'Knock Outs', and Cancer. CNIO Cancer Conference: Cancer and Aging. November 07 -09, 2005, Madrid, Spain.

Wang Z, Kineman RD, Luque RM, Shirai T, Lantvit DD, Unterman TG, Prins GS, Swanson SM. Growth ormone receptor mRNA is upregulated, while IGF-I receptor mRNA is downregulated during prostate carcinogenesis in the Probasin/SV40TAg rat. 88th Annual Meeting of the Endocrine Society, June 24-27, 2006, Boston, MA.

Conclusions

We have established new animal models of prostate cancer to test the hypothesis that GH signaling is required for prostate carcinogenesis. Our mouse model demonstrated that the loss of GHR produced a significant reduction in the level of PIN in the ventral as well dorsal-lateral lobes in terms of incidence and PIN area. We have also found that disruption of GH signaling also retards prostate carcinogenesis induced by the powerful Tag oncogene in the rat model. These results would support the initiation of clinical trials for novel therapeutics that modulate this pathway. These new animal models could also be used to ask questions regarding the mechanisms by which growth hormone and IGF-I regulate growth and differentiation of the prostate gland and modulate susceptibility of the prostate to oncogenic agents. For example, what are the differences between tumors (if any) that can develop in the absence of a functional GH/IGF axis? Are there differences in androgen dependence? The basic tools needed to develop the animal models to address these questions can be developed given what is currently available.

Reference

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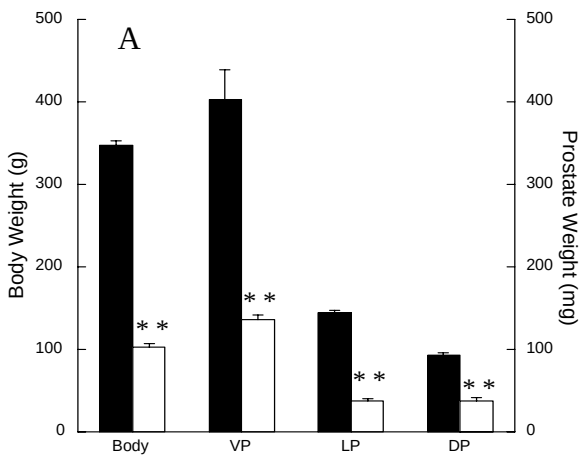
Appendix 1: Figures

FIGURE 1


A
Body Weight (g)
Prostate Weight (mg)
500
400
300
200
100
0
* *
* *
* *
* *
Body
VP
LP
DP

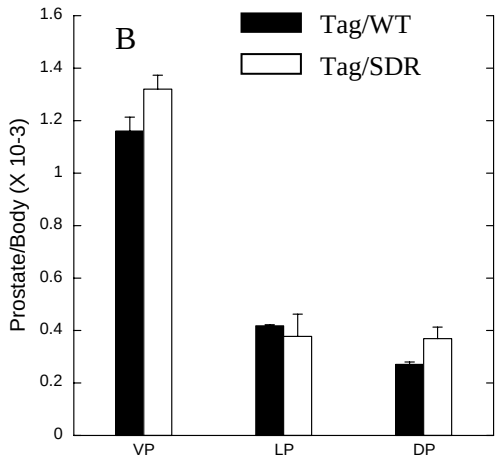

B
Tag/WT
Tag/SDR
Prostate/Body (X 10-3)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
VP
LP
DP

FIGURE 2

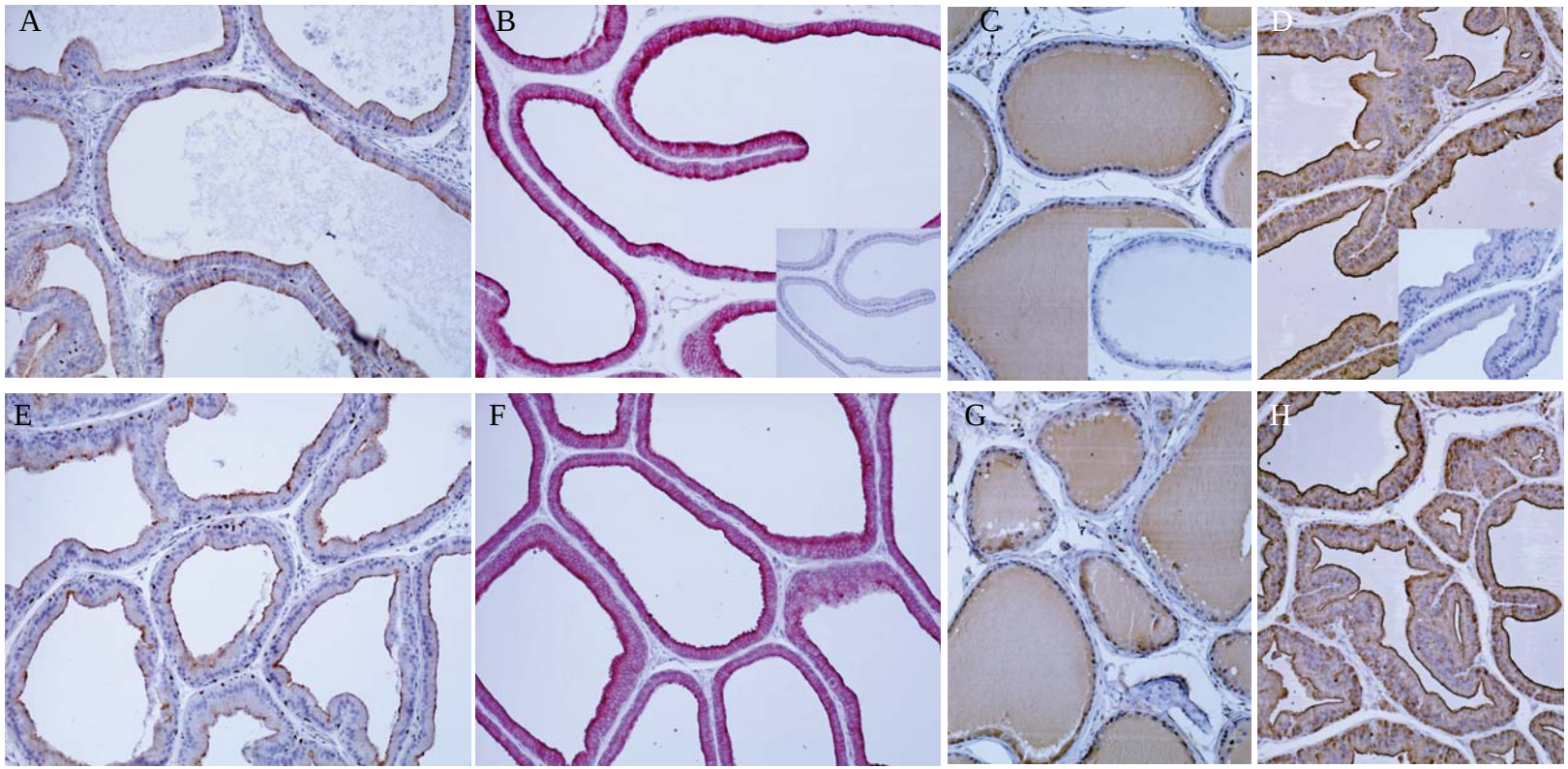


FIGURE 3

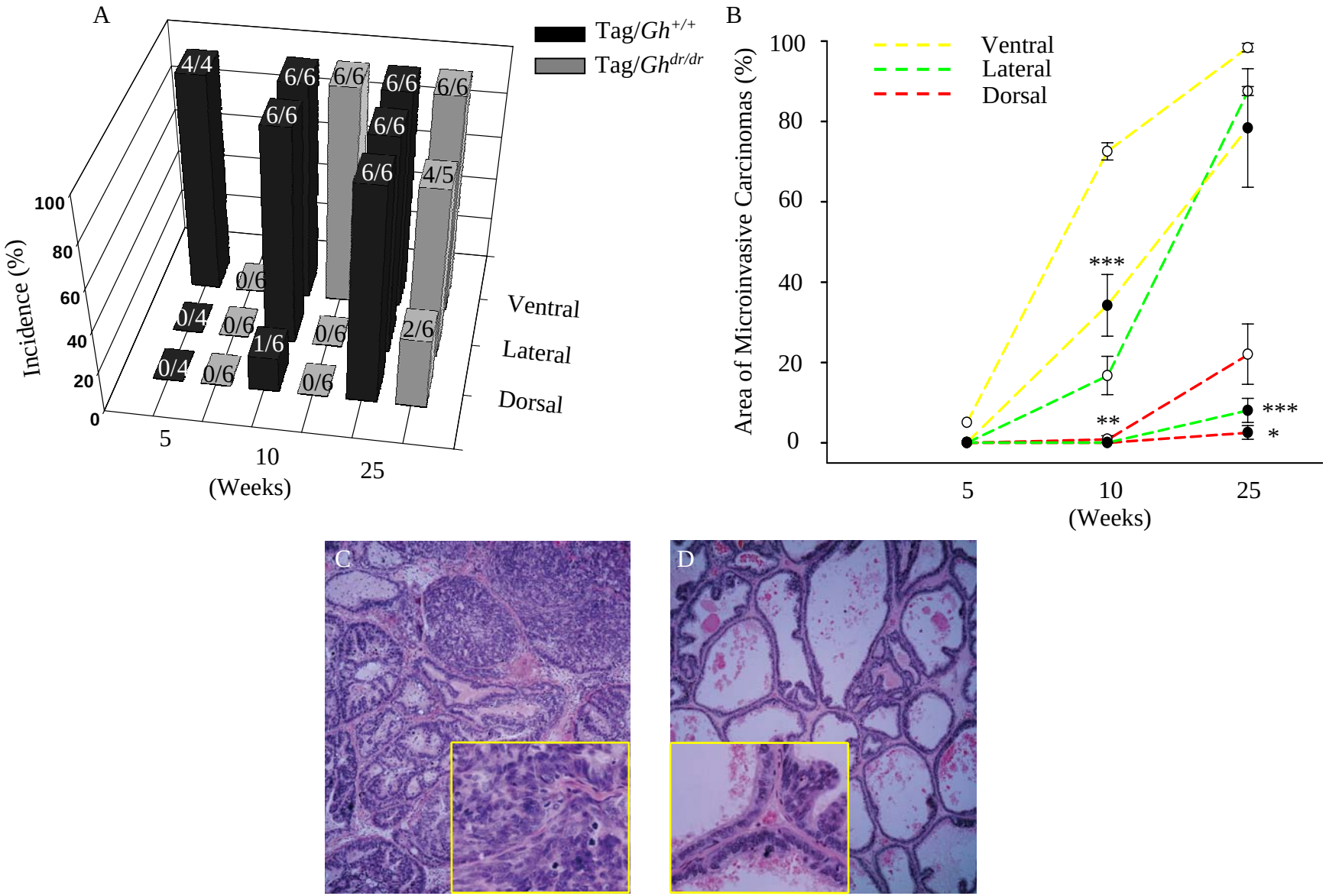
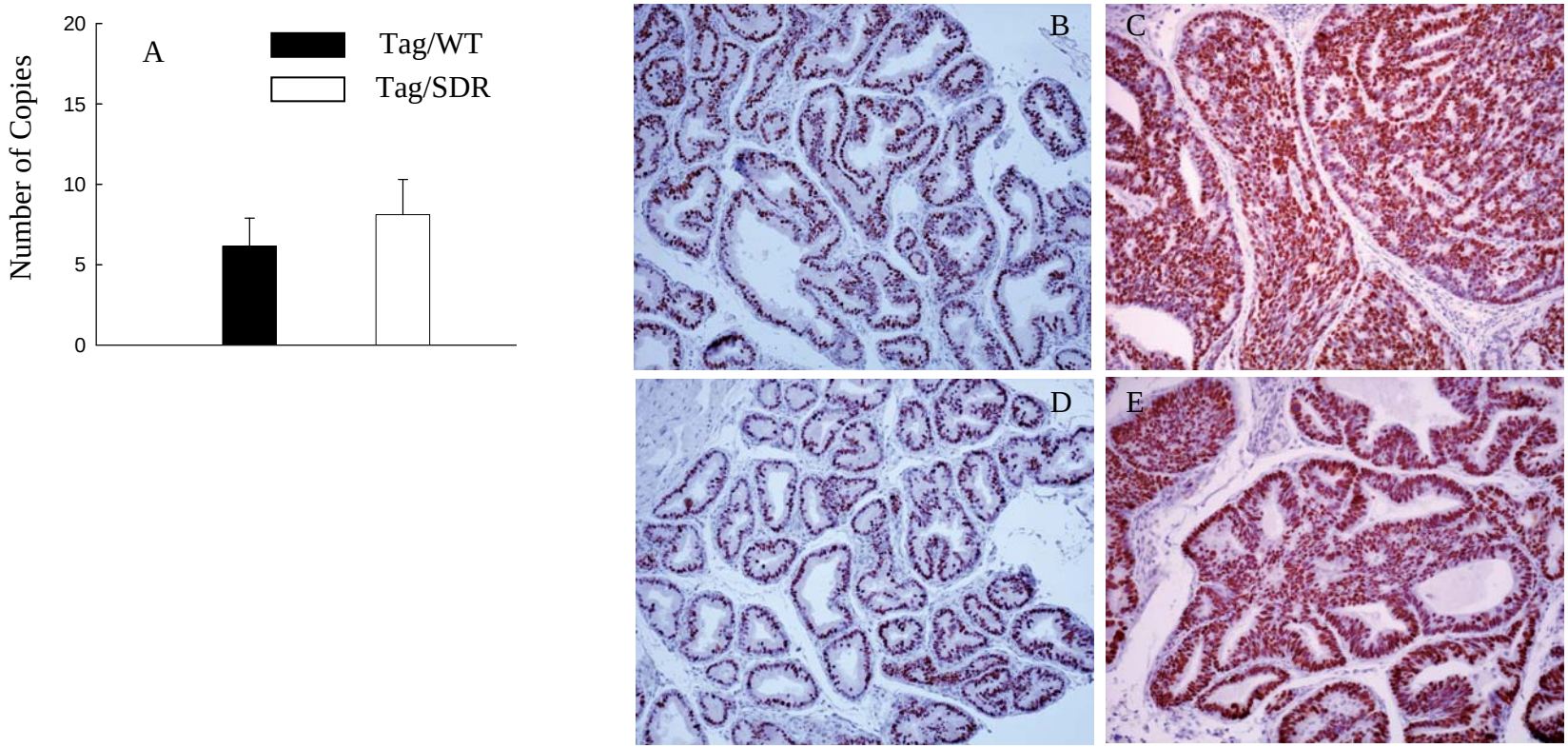

A
Tag/Gh+/+
Tag/Ghdr/dr
Incidence (%)
100
80
60
40
20
0
4/4
6/6
6/6
6/6
6/6
6/6
6/6
6/6
4/5
0/6
0/4
0/6
1/6
0/6
2/6
0/4
0/6
0/6
Ventral
Lateral
Dorsal
5
10
25
(Weeks)
B
Area of Microinvasive Carcinomas (%)
100
80
60
40
20
0
Ventral
Lateral
Dorsal
***
**
***
*
5
10
25
(Weeks)
C
D

FIGURE 4



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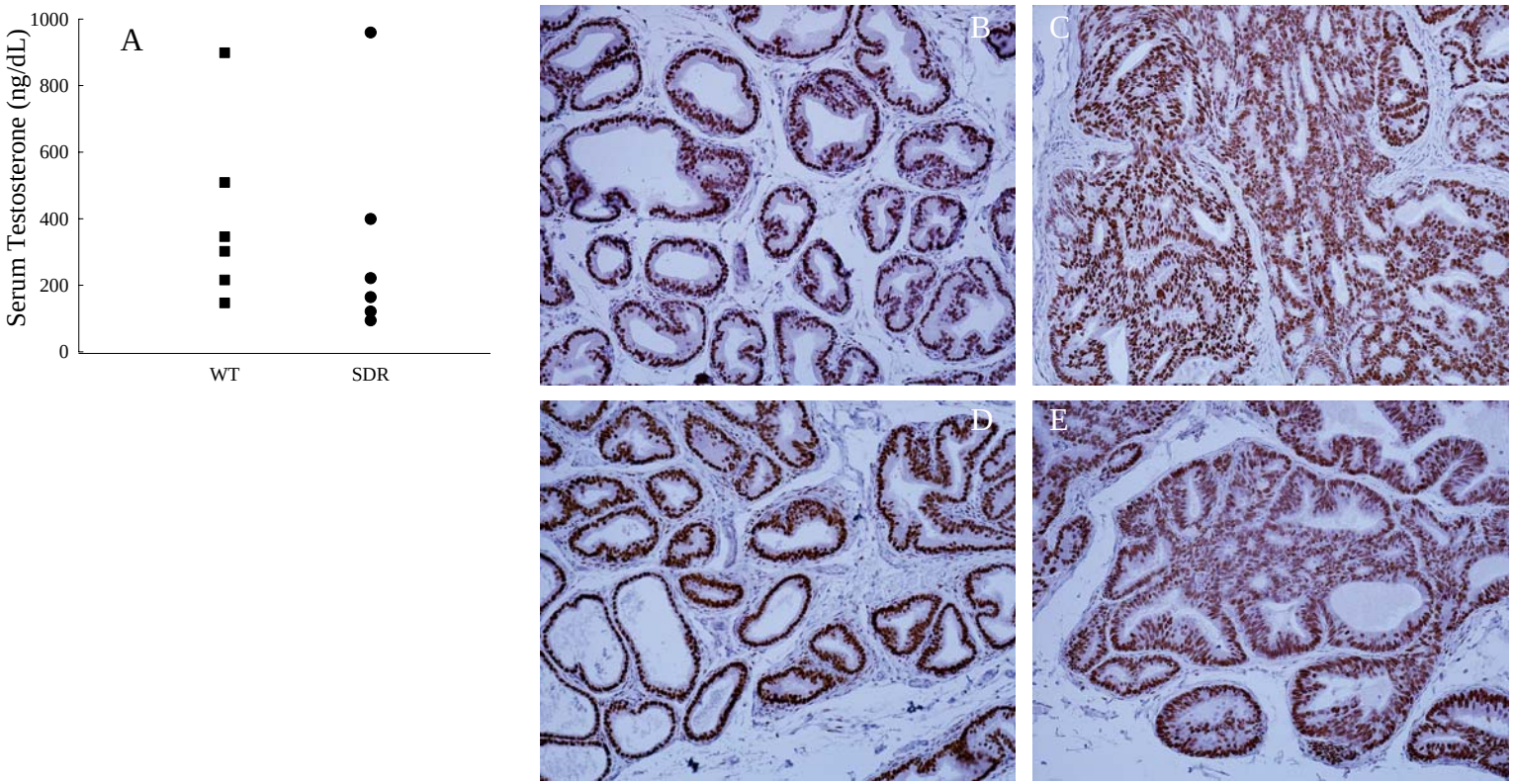


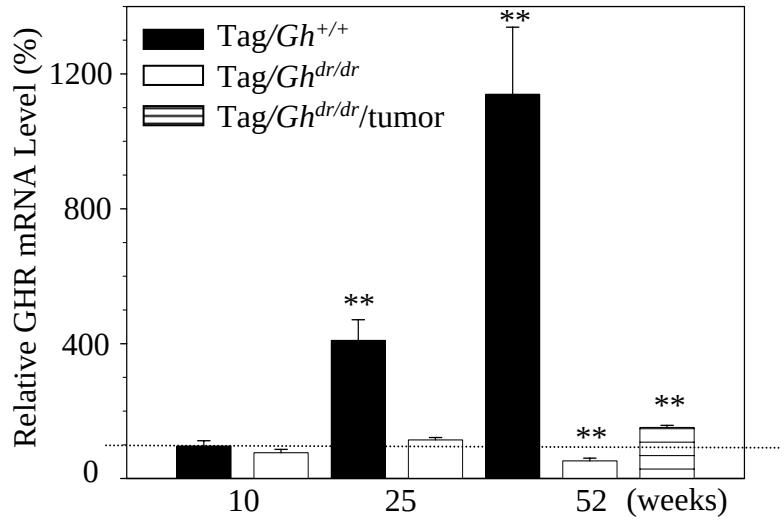

Relative GHR mRNA Level (%)
1200
800
400
0
Tag/Gh+/+
Tag/Ghdr/dr
Tag/Ghdr/dr/tumor
**
**
**
**
10
25
52
(weeks)

FIGURE 6

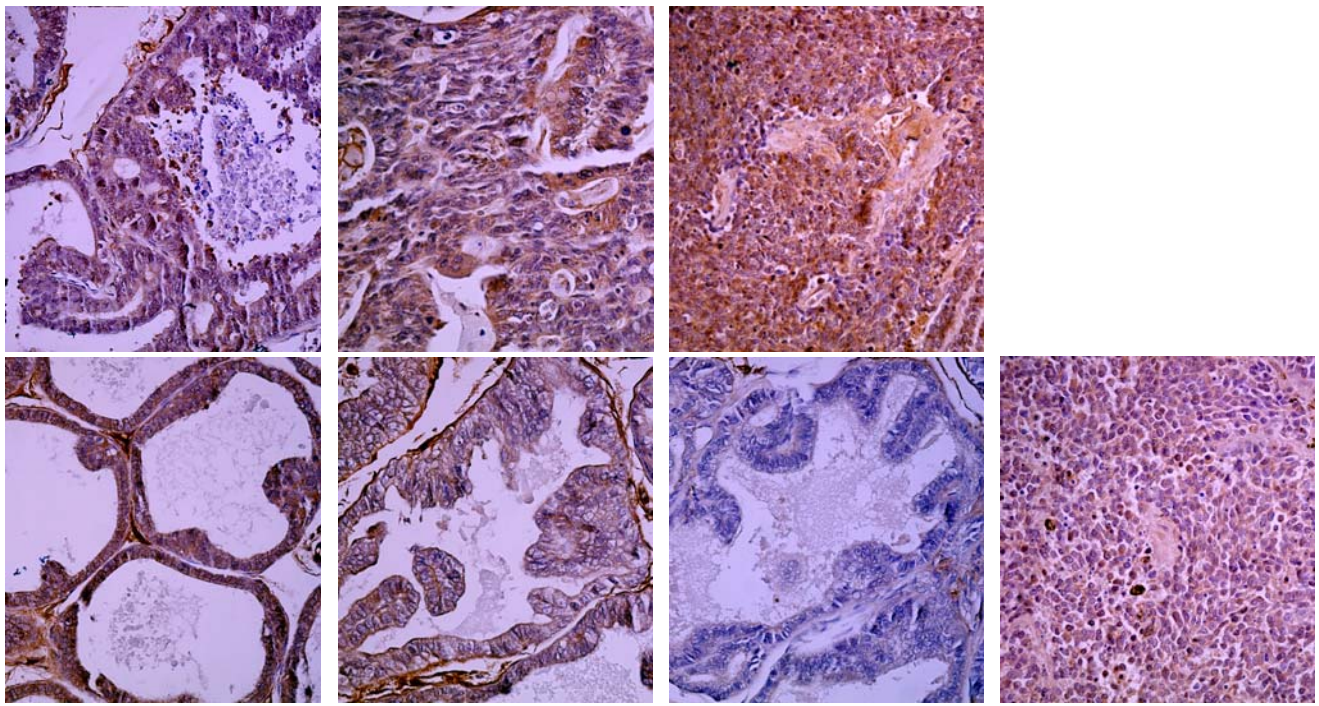
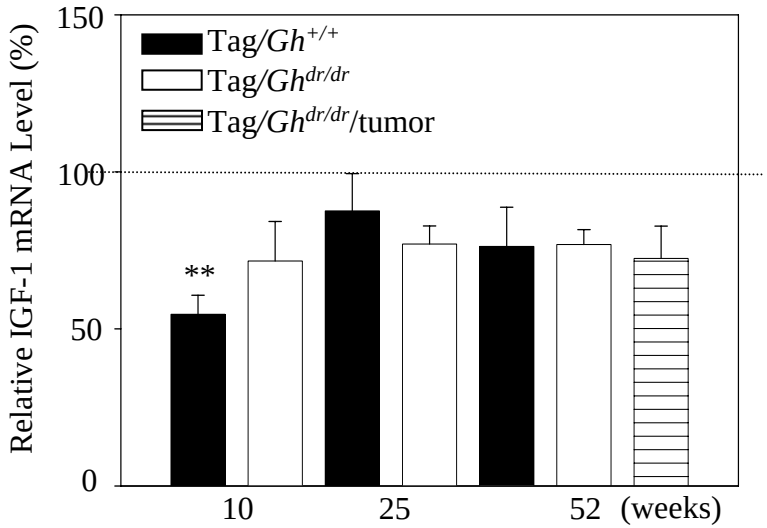
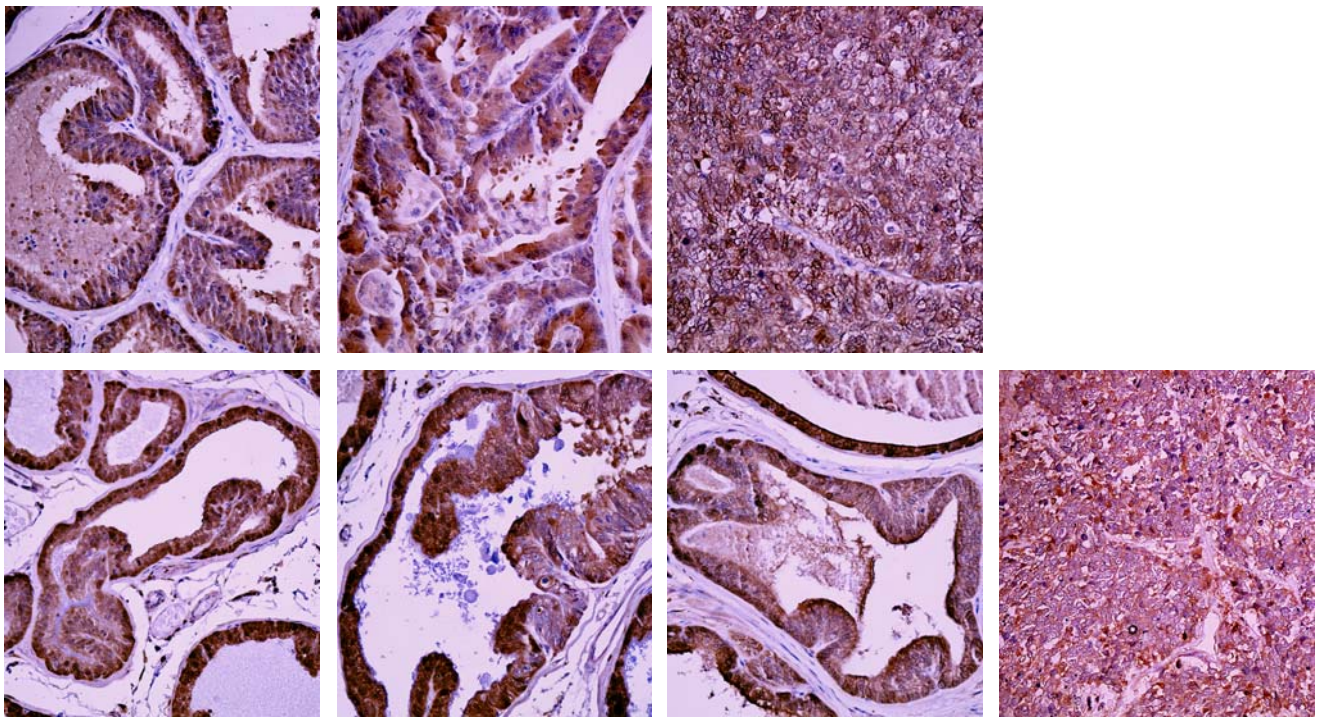


FIGURE 7


150
100
50
0
Relative IGF-1 mRNA Level (%)
Tag/Gh+/+
Tag/Ghdr/dr
Tag/Ghdr/dr/tumor
**
10
25
52
(weeks)



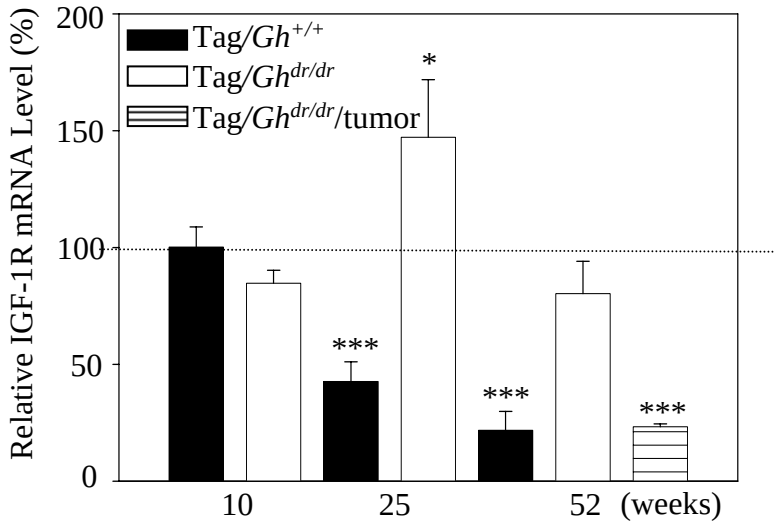

Relative IGF-1R mRNA Level (%)
200
150
100
50
0
Tag/Gh+/+
Tag/Ghdr/dr
Tag/Ghdr/dr/tumor
*
***
***
***
10
25
52
(weeks)

FIGURE 8

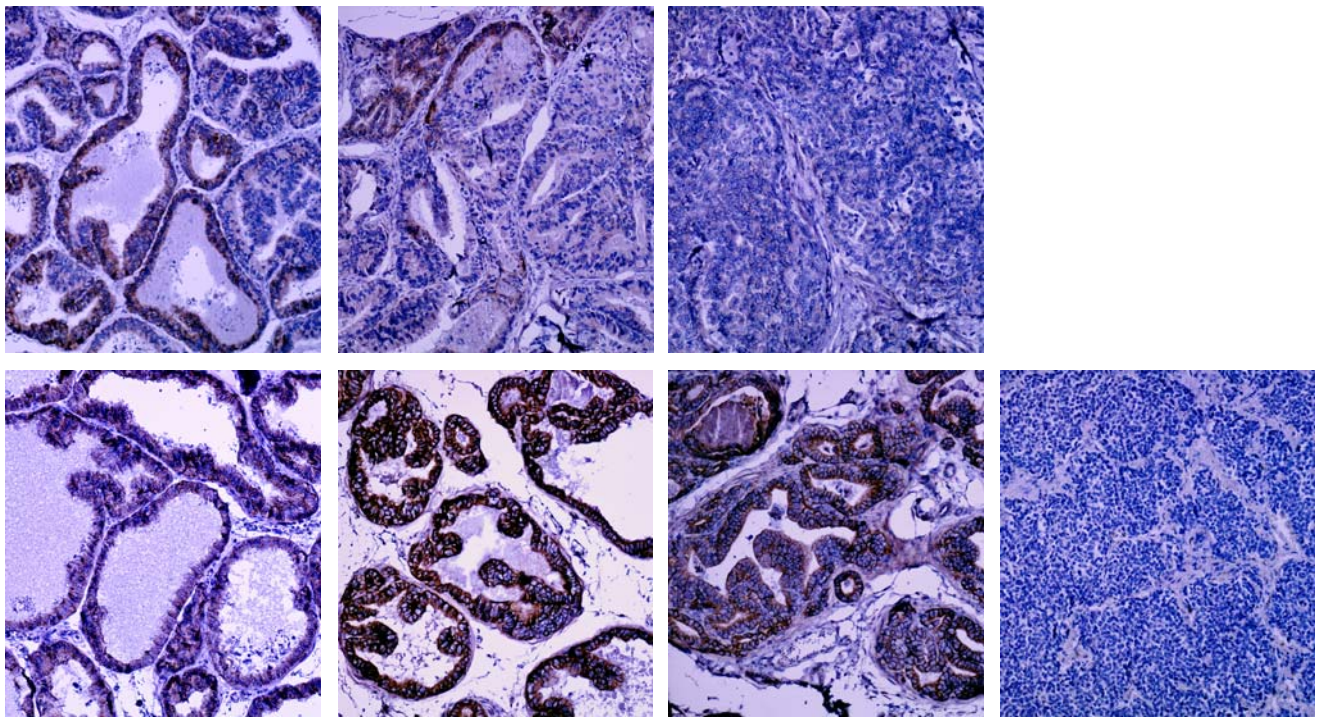
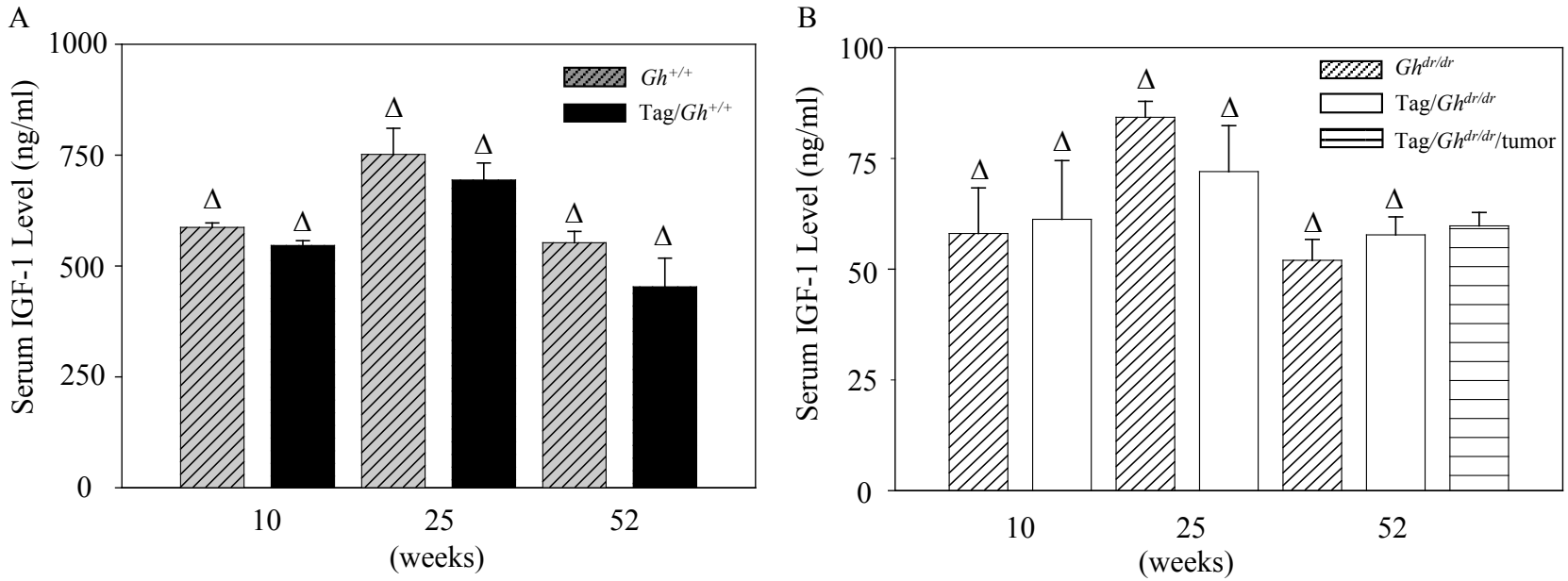


FIGURE 9


A
1000
750
500
250
0
Serum IGF-1 Level (ng/ml)
Gh+/+
Tag/Gh+/+
Δ
10
25
52
(weeks)
B
100
75
50
25
0
Serum IGF-1 Level (ng/ml)
Ghdr/dr
Tag/Ghdr/dr
Tag/Ghdr/dr/tumor
Δ
10
25
52
(weeks)

Appendix 2:

Manuscript describing completed objectives of Task 1

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Disruption of Growth Hormone Signaling Retards Early Stages of Prostate Carcinogenesis in the C3(1)/T Antigen Mouse

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Recent epidemiological studies suggest that elevated serum titers of IGF-I, which are, to a large degree, regulated by GH, are associated with an increase in prostate cancer risk. The purpose of the current study was to develop the first animal models to directly test the hypothesis that a normal, functional GH/IGF-I axis is required for prostate cancer progression. The GH receptor (GHR) gene-disrupted mouse ($Ghr^{-/-}$), which has less than 10% of the plasma IGF-I found in GHR wild-type mice, was crossed with the C3(1)/T antigen (Tag) mouse, which develops prostatic intraepithelial neoplasia driven by the large Tag that progress to invasive prostate carcinoma in a manner similar to the process observed in humans. Progeny of this cross were genotyped and Tag/$Ghr^{+/+}$ and Tag/$Ghr^{-/-}$ mice were killed at 9 months of age. Seven of eight Tag/$Ghr^{+/+}$ mice harbored prostatic intraepithelial neoplasia lesions of various grades. In contrast, only one of the eight Tag/$Ghr^{-/-}$ mice exhibited atypia ($P < 0.01$, Fischer's exact test). Disruption of the GHR gene altered neither prostate androgen receptor expression nor serum testosterone titers. Expression of the Tag oncogene was similar in the prostates of the two mouse strains. Immunohistochemistry revealed a significant decrease in prostate epithelial cell proliferation and an increase in basal apoptotic indices. These results indicate that disruption of GH signaling significantly inhibits prostate carcinogenesis. (*Endocrinology* 146: 5188–5196, 2005)

PROSTATE CANCER IS the most common and second deadliest form of cancer afflicting American men (1). Androgens are important regulators of prostate proliferation, differentiation, and apoptosis, and androgen antagonism remains the primary treatment for prostate cancer. Although initially effective, most patients' tumors reemerge as androgen-independent disease. Clearly, other treatment modalities are urgently needed. Recent clinical and epidemiologic studies suggest that GH and IGF-I are important for normal human prostate growth as well as prostate cancer. For example, chronic GH deficiency in adulthood is associated with reduced prostate volume (2). The prevalence of prostate hyperplasia has been reported to be lower in GH-deficient patients than controls (3). Also, acromegalic subjects are known to have enlarged prostates that decrease significantly in size on treatment with somatostatin analogs or surgery to lower GH levels (4).

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Abbreviations: AR, Androgen receptor; CK18, cytokeratin 18; GHR, GH receptor; mDLP, mouse dorsolateral prostate; PCNA, proliferating cell nuclear antigen; PIN, prostatic intraepithelial neoplasia; Tag, T antigen; TUNEL, terminal deoxynucleotide-transferase-mediated deoxyuridine 5-triphosphate-digoxigenin nick end labeling; VP, ventral prostate.

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Many of the biological activities of GH are mediated by IGF-I. IGF-I is unique among growth factors in that it is also an endocrine hormone. GH stimulates IGF-I production in the liver and peripheral tissues. In the blood, IGFs are bound to high-affinity IGF binding proteins that serve as both carrier proteins and modulators of IGF bioactivity. The major IGF binding protein is IGF binding protein-3, which accounts for more than 75% of the bound IGF in the circulation. The endocrine aspect of IGF physiology has facilitated epidemiologic studies on the relationship of circulating levels of IGF-I and their binding proteins to cancer risk.

A number of epidemiologic studies have been conducted to evaluate the role of the GH/IGF axis in prostate carcinogenesis (5). Whereas three prospective studies found a positive association between serum IGF-I and prostate cancer risk (6–8), one prospective study reported an inverse relationship (9). Case-control studies are also divided, with some studies suggesting that elevated serum IGF-I is associated with increased prostate cancer risk (10–16), whereas others find little or no association (17–21). Both metaanalyses of the literature conducted to date agree that there is a positive association between serum IGF-I level and prostate cancer risk (22, 23). With regard to GH, a recent case-control study suggests that elevated basal GH serum titers lowered prostate cancer risk (24). Therefore, whereas epidemiologic studies suggest that the GH/IGF axis may influence carcinogenesis in the prostate, more work is needed to clarify this issue.

Animal models in which hormone signaling can be better controlled may be helpful in determining the role of the GH/IGF axis in prostate carcinogenesis.

The GH/IGF system has proven to be important in regulating proliferation of cancer cells in laboratory-based studies. Human prostate cancer cell lines such as LNCaP and PC3 express GH receptors (GHRs) at levels greater than observed in normal tissue (25). Pollak *et al.* (26) reported that the growth of androgen-independent PC3 cells is slowed in GH-deficient Little mice ($Ghrhr^{lit/lit}$) relative to control mice. Schally *et al.* (27) published studies recently that demonstrate an inhibitory effect of GHRH antagonists on the growth of human prostate cancer cells, including androgen-independent lines, in immunodeficient mice. For example, these investigators reported that the GHRH antagonist MZ-4-71 decreased IGF-I levels in not only serum of treated animals but also the tumors (28). These studies suggest that the GH/IGF axis is important for the growth of advanced human prostate cancers.

Whereas previous laboratory-based studies have shown that disruption of the GH/IGF axis can inhibit the proliferation of advanced human prostate cancers propagated either *in vitro* as cell cultures or *in vivo* as xenografts in immunodeficient mice, we asked in the present studies whether the GH/IGF axis plays a role in the progression of prostate cancers from initiated cells to preneoplastic lesions. Given the role that the GH/IGF-I axis plays in regulating prostatic cell proliferation and differentiation and prostate gland growth and differentiation, we hypothesized that an intact GH/IGF-I axis is required for prostate cancer cells harboring the T antigen (Tag) oncogene to progress to prostatic intraepithelial neoplasia (PIN). Our approach was to cross the Laron mouse, in which the gene coding for both GHR and GH binding protein has been disrupted or knocked out, with the C3(1)/Tag mouse, which develops prostate cancers due to the Tag oncogene it harbors. We chose the C3(1)/Tag mouse from the many available transgenic models of prostate cancer because it develops prostate cancer relatively slowly, and disease progression from low-grade PIN through high-grade PIN to invasive carcinoma is well characterized (29). This aspect of the model makes it particularly well suited for studies on the prevention of prostate cancer progression.

Materials and Methods

Animals

All studies involving animals were conducted in accordance with mandated standards of humane care as stipulated in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (30). Furthermore, the Institutional Animal Care and Use Committee of the University of Illinois at Chicago approved all experimental protocols involving animals before the initiation of any procedures. All the animals were bred at the Biologic Resources Laboratory (University of Illinois at Chicago). They were fed Teklad 8640 diet (Harlan Teklad, Madison, WI) and given water *ad libitum* and housed in a temperature- and humidity-controlled environment with a regular light/dark illumination cycle (lights on at 0600 h and off at 1900 h).

The C3(1)/Tag transgenic mouse was developed by Green and colleagues (31) in an FVB/N background. The transgene includes the early region of simian virus 40 with the large tumor antigen. Expression is

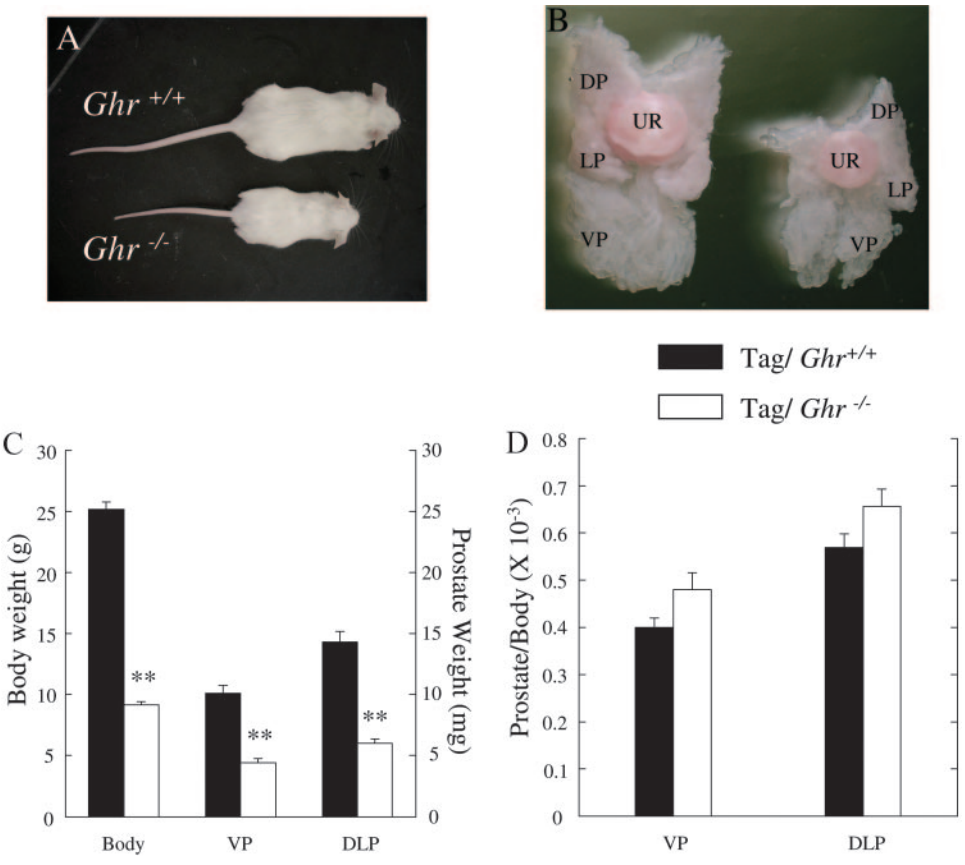

FIG. 1. Gross anatomy of the Tag/$Ghr^{+/+}$ *vs.* the Tag/$Ghr^{-/-}$ mouse prostate. A, Comparison of littermates at 9 wk of age. B, The VP and dorsolateral (DLP) lobes of the prostate are shown attached to a section of the urethra (UR). C, Body and lobe weights of Tag/$Ghr^{-/-}$ mice are distinguished from controls (n = 10 in each group). D, The lobes to body weight ratios for comparison between Tag/$Ghr^{+/+}$ and Tag/$Ghr^{-/-}$ are shown. Although the Tag/$Ghr^{-/-}$ mice were less than half the size and weight of wild-type littermates, the prostate weight to body weight ratio was not significantly different between the two genotypes ($P > 0.05$, *t* test). **, Significantly different from Tag/$Ghr^{+/+}$ control ($P < 0.01$).

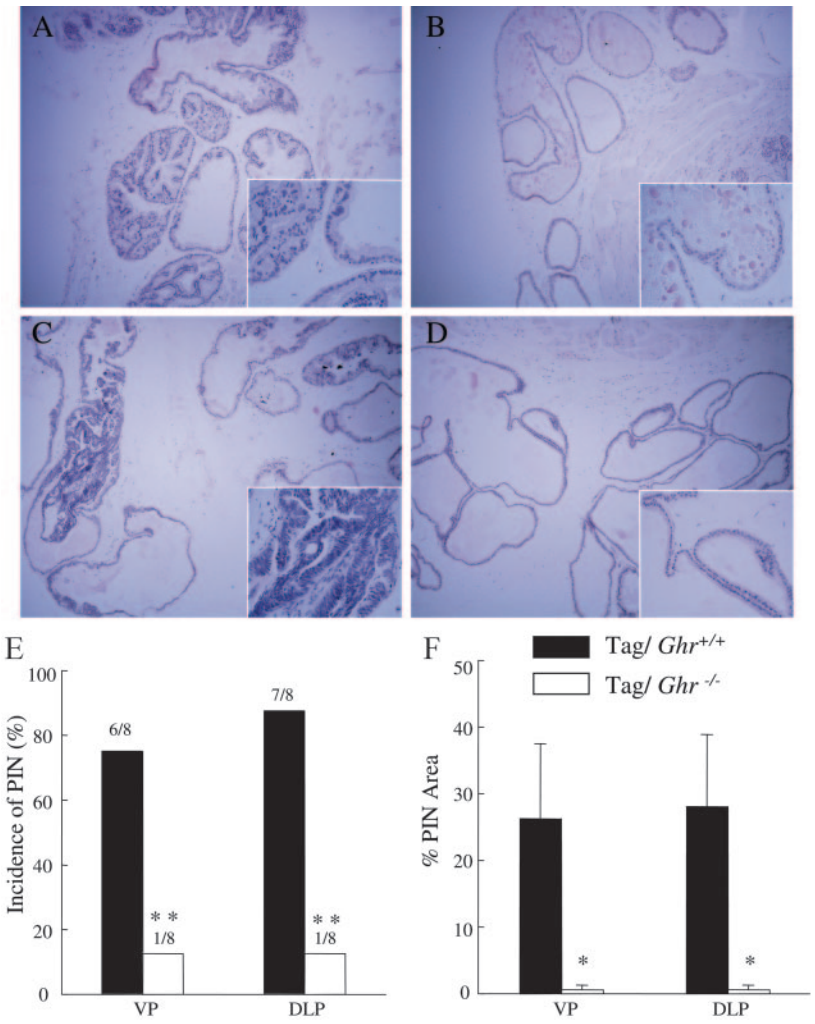

FIG. 2. Prostate histopathology in adult Tag/$Ghr^{+/+}$ and Tag/$Ghr^{-/-}$ mice. A–D, Representative hematoxylin and eosin-stained sections of each lobe and genotype from 38-wk-old mice. Original magnification for A–D was ×10 and the *insets*, which highlight areas within the larger panels, was ×40. A illustrates an example of a PIN lesion in transition from low-grade to high-grade PIN in the dorsolateral prostate (DLP) of a Tag/$Ghr^{+/+}$ mouse. C is an example of a high-grade PIN in the VP of a Tag/$Ghr^{+/+}$ mouse. B and D illustrate normal epithelium in the dorsolateral and ventral lobes of Tag/$Ghr^{-/-}$ mice, which was the predominant phenotype in this mouse. Incidences of PIN lesions in the two genotypes are shown with the number of mice examined in E (*i.e.* 6/8 means six of eight animals harbored PIN). The area of these lesions was measured using MetaVue image analysis software and expressed as a percentage of total prostate tissue area (F). *, Significantly different from Tag/$Ghr^{+/+}$ control ($P < 0.05$); **, $P < 0.01$.

targeted to the prostate by the 5′-flanking region of the rat *C3(1)* gene. By 8 wk of age, male mice develop foci of PIN that appear identical with human PIN (29).

The GHR and GH binding protein are encoded by a single GHR/binding protein gene in mammalian species (32). Homozygous GHR/binding protein knockout mice (referred to here as $Ghr^{-/-}$) display postnatal growth retardation, proportionate dwarfism, and absence of the GHR and binding protein. Serum GH levels for $Ghr^{-/-}$ mice are greatly elevated, compared with either $Ghr^{+/+}$ or $Ghr^{+/-}$ mice (33). Serum IGF-I levels in $Ghr^{-/-}$ mice are decreased by about 90%, compared with $Ghr^{+/+}$ and $Ghr^{+/-}$ mice (34). No other abnormalities are evident in the homozygous or heterozygous knockout mice; their behavior is indistinguishable from that of their wild-type littermates, and lactation in $Ghr^{+/-}$ mice is adequate to feed their young.

For the current studies, FVB mice heterozygous for the C3(1)/Tag transgene were crossed with $Ghr^{-/-}$ mice of a BALB/c background. Genotyping was conducted by PCR as previously described (34). Offspring of this initial cross were used to generate mice for the current studies that carried the C3(1)/Tag transgene in the presence (Tag/$Ghr^{+/+}$) or absence (Tag/$Ghr^{-/-}$) of a wild-type GHR gene.

Histopathology

Male Tag/$Ghr^{+/+}$ mice and Tag/$Ghr^{-/-}$ mice were killed by CO_2 asphyxiation at 38 wk of age. The entire genitourinary bloc (prostate lobes, seminal vesicles ampullary glands, proximal ductus deferens, bladder, and proximal urethra), were excised and fixed in 10% neutral buffered formalin. The lobes of prostates were dissected with the aid of a dissecting microscope and were embedded in paraffin. Two sets of sections, each consisting of at least 15 serial sections, were cut from each block. The sets of sections were separated by at least 100 μm within each block. Four-micrometer sections were placed on SuperFrost/Plus slides (Fisher Scientific Co., Pittsburgh, PA) and stained with hematoxylin and eosin, visualization of dorsal prostate, lateral prostate, and ventral prostate (VP). The slides were read by a board-certified pathologist and a second pathologist experienced in rodent prostate pathology. Both pathologists were blinded to the genotype of the specimens. The dorsolateral and ventral lobes were separately analyzed for absence or presence of hyperplasia, nuclear atypia characterized as PIN, microinvasion, and tumors according to criteria established by the Mouse Models of Human Cancer Consortium Prostate Cancer Committee (35). The observed PIN lesions were further divided into two stages, low grade and high grade, as described by Shibata *et al.* (29). Finally, the area of PIN lesions within each specimen was measured using image analysis software (MetaVue, Universal Imaging, Downingtown, PA).

Immunohistochemistry

Expression of androgen receptor (AR), Tag, p63, cytokeratin 18 (CK18), mouse dorsolateral prostate (mDLP) and proliferating cell nuclear antigen (PCNA) were evaluated by immunohistochemistry following protocols by the respective antibody vendors or literature (36). Tissues were fixed and embedded using methods described above for histopathology. Paraffin sections were heat immobilized (60 C, 1 h), deparaffinized in three changes of xylene, hydrated in a series of graded ethanol, and rinsed in several changes of distilled water. Heat-induced antigen retrieval was performed in either a microwave oven (Tag) or a pressure cooker (p63, CK18, mDLP, AR, and PCNA; decloaking cham-

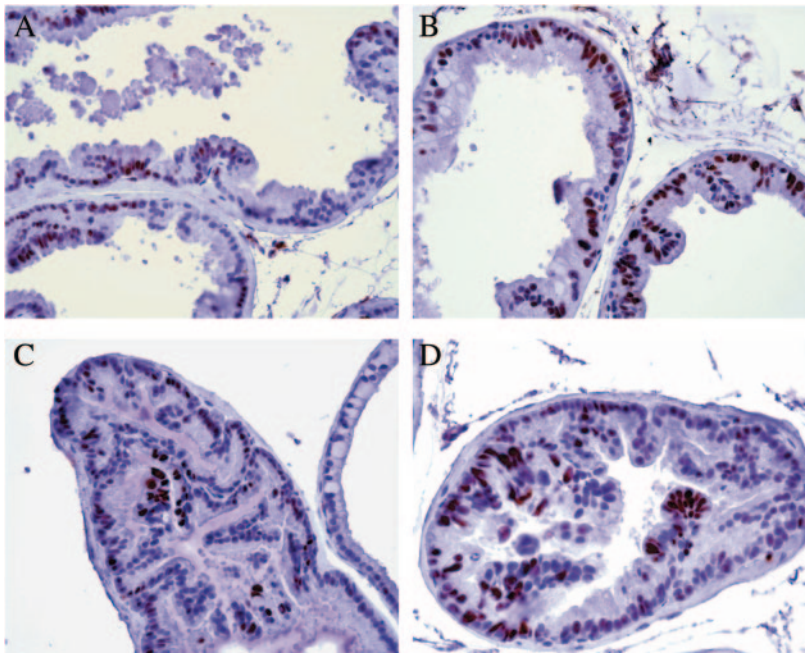

FIG. 3. Nuclear expression of Tag protein in Tag/$Ghr^{+/+}$ (A and C) and Tag/$Ghr^{-/-}$ (B and D) mice at 38 wk of age. Prostates were excised and fixed in formalin and embedded in paraffin before sectioning. Immunohistochemical analysis of Tag expression revealed no significant difference in either low-grade PIN (*top panels*) or high-grade PIN (*lower panels*) in both genotypes.

ber; Biocare Medical, Concord, CA). Endogenous peroxidase was quenched using H_2O_2 (3%, 10 min). After blocking with the appropriate serum, the prostate sections were treated with mouse anti-Tag (1:50, PAb101; BD PharMingen, San Diego, CA), rabbit anti-p63 (1:500, H137; Santa Cruz Biotechnology, Santa Cruz, CA), sheep anti-CK18 (1:200, PH504; Binding Site, Birmingham, UK), rabbit anti-mDLP (1:5000), rabbit anti-AR (2 μg/ml, PG21), or mouse anti-PCNA (1:80, PC-10; Oncogene Research Products, San Diego, CA) and sequentially with secondary antibodies and Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) for rabbit and sheep primary antibody or Vector M.O.M kit (Vector) for mouse primary antibodies. Sections were rinsed in PBS and incubated with 3,3′-diamobenzidine (Sigma Chemical, St. Louis, MO). Slides were counterstained in hematoxylin, dehydrated in graded ethanol, cleared in xylene, and mounted using Permount mounting medium. Tissue specimens from each genotype were processed together to eliminate interassay variability as a confounding factor in analysis. Apoptosis was assessed by the terminal deoxynucleotide-transferase-mediated deoxyuridine 5-triphosphate-digoxigenin nick end labeling (TUNEL) assay using ApopTag apoptosis detection systems (Serologicals Corp., Norcross, GA) according to the manufacturer's protocol. To compare the cell proliferation and apoptosis levels in the Tag/$Ghr^{+/+}$ mice and Tag/$Ghr^{-/-}$ mice, the number of PCNA-immunoreactive cells staining positively in the nucleus per 1000 cells were scored in normal-appearing prostate epithelium. Sampling was done by two independent pathologists who randomly selected fields of normal prostate to score. Both investigators were blinded as to the genotype of the specimens.

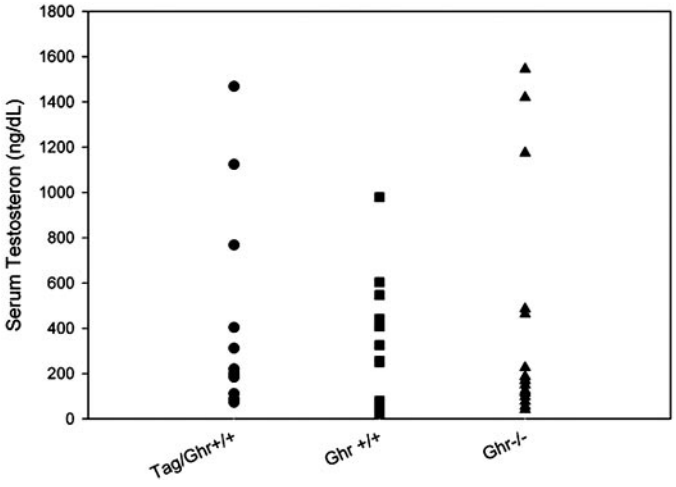

FIG. 4. Serum testosterone levels in $Ghr^{+/+}$, $Ghr^{-/-}$, and Tag/$Ghr^{+/+}$ mice. Serum testosterone was measured by RIA in adult (19–22 wk) male mice (n = 12–16 per group). Testosterone levels were not affected by either Tag (Tag/$Ghr^{+/+}$ *vs.* $Ghr^{+/+}$) or GHR ($Ghr^{+/+}$ *vs.* $Ghr^{-/-}$) expression status as determined by ANOVA.

Serum testosterone

For each group of adult (19–22 wk of age) male $Ghr^{+/+}$, $Ghr^{-/-}$, or Tag/$Ghr^{+/+}$ mice, blood was obtained from the aorta under ketamine/xylazine anesthesia. Serum samples were stored at −20 C until RIA for testosterone (Coat-A-Count total testosterone; Diagnostic Products Corp., Los Angeles, CA).

Statistical analysis

All the data are presented as means ± SEM. The significance of intergroup differences in serum hormone levels, cell proliferation levels, and apoptosis levels were analyzed using one-way ANOVA, two-tailed *t* test, or two-sided individual *t* test, respectively, unless otherwise indicated.

Results

Characteristics of experimental animals

As expected, body weight and length were reduced in Tag/$Ghr^{-/-}$, compared with age-matched Tag/$Ghr^{+/+}$ mice, at 9 wk of age (n = 10, Fig. 1, A and C). The seminal vesicles, coagulating gland (data not shown), ventral prostate, and dorsolateral prostate were all present and reduced in size but of normal appearance in Tag/$Ghr^{-/-}$ mice, compared with Tag/$Ghr^{+/+}$ mice at 9 wk of age (Fig. 1B) and in mature animals of 38 wk of age (not shown). The average ventral prostate and dorsolateral prostate weights were significantly lower ($P < 0.0001$) in Tag/$Ghr^{-/-}$ mice than in Tag/$Ghr^{+/+}$ mice (Fig. 1C). However, no significant difference in the prostate to body weight ratio was observed between Tag/

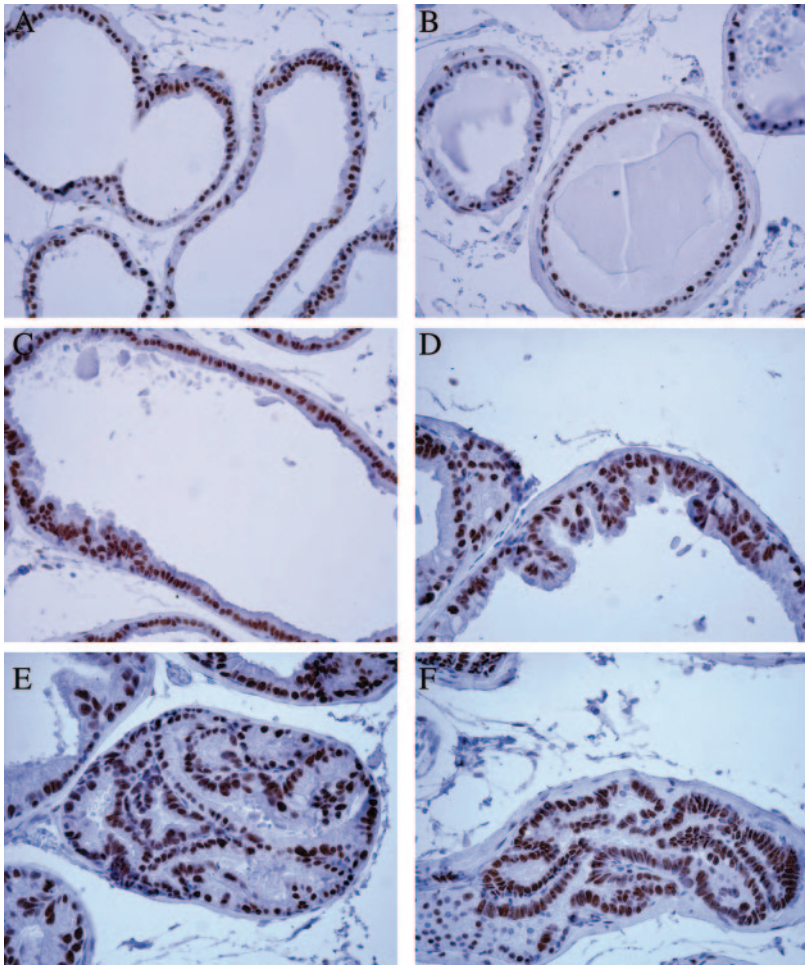

FIG. 5. Disruption of the GHR gene does not affect murine AR expression. Prostates were excised from 38-wk-old mice and processed for immunohistochemical analysis. AR expression was similar in normal prostate epithelial cells (A and B), low-grade-PIN (C and D), and high-grade PIN (E and F) in both Tag/Ghr$^{+/+}$ (A, C, and E) and Tag/*Ghr*$^{-/-}$ (B, D, and F) mice.

Ghr$^{-/-}$ mice and Tag/*Ghr*$^{+/+}$ mice (Fig. 1D), indicating that the reduction in prostate weight is proportionate to the reduction in body weight, consistent with an effect of reduced GH action.

Prostate carcinogenesis is blocked by disruption of the GHR

Mice were killed at 38 wk of age, and their prostates were dissected as described in *Materials and Methods*. This time point was chosen because previous studies indicate that male C3(1)/Tag mice develop prostate cancers beginning at 7 months of age and that by 8 months of age, the majority of mice had developed prostate cancers. Killing the mice at 38 wk of age (about 9.5 months) was chosen for the current studies in the hope that all control mice would have developed prostate cancers by this time point. The serial sections of prostate lobes were examined histologically for PIN lesions as described previously (29) (Fig. 2, A–D). Seven of eight Tag/*Ghr*$^{+/+}$mice harbored PIN lesions of various grades in the dorsolateral and ventral lobes (Fig. 2, A and C). For the dorsolateral lobes, two had low-grade PIN, four had combined low-grade PIN and high-grade PIN lesions, and one had exclusively high-grade PIN. For the ventral lobes, three had low-grade PIN and three had combined low-grade PIN and high-grade PIN lesions. In contrast, of the eight Tag/*Ghr*$^{-/-}$ mice, only one harbored low- and high-grade PIN lesions, taking up 5% of the prostate (Fig. 2, B and D). This change in incidence was highly significant ($P < 0.01$) as determined by Fischer's exact test (Fig. 2E). The area of PIN was also significantly higher ($P < 0.04$) in all lobes of Tag/*Ghr*$^{+/+}$ than in Tag/*Ghr*$^{-/-}$ mice (Fig. 2F). However, no tumors were detected in either group of animals.

Expression of Tag

Tag/*Ghr*$^{+/+}$ and Tag/*Ghr*$^{-/-}$ mice harbored few cells in normal-appearing prostate epithelium that stained positive for Tag. However, the number of immunoreactive epithelial cells increased progressively from low-grade PIN (Fig. 3, A and B) to high-grade PIN (Fig. 3, C and D), which is consistent with the findings of Shibata *et al.* (29) for the original C3(1)/Tag mouse. When Tag expression was compared between PIN lesions of similar severity, no difference in the degree of Tag expression was observed between Tag/*Ghr*$^{+/+}$ mice and Tag/*Ghr*$^{-/-}$ mice (Fig. 3). Therefore, the lack of the GHR in this model does not appear to affect Tag expression in prostate epithelium.

Neither Tag expression nor disruption of GH signaling alters testosterone levels or AR expression

Serum testosterone levels were analyzed in groups of adult (19–22 wk) male *Ghr*$^{+/+}$, *Ghr*$^{-/-}$, and Tag/*Ghr*$^{+/+}$

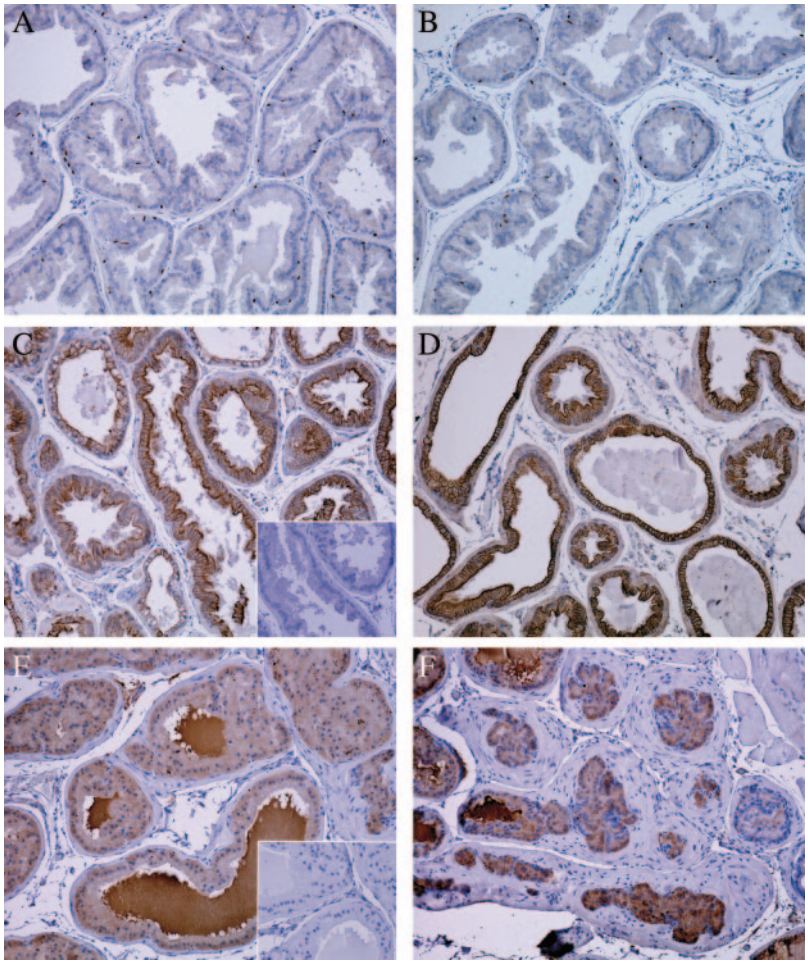

FIG. 6. Expression of prostate differentiation markers is similar between Tag/$Ghr^{+/+}$ and Tag/$Ghr^{-/-}$ mice. Prostate tissue sections from Tag/$Ghr^{+/+}$ (A, C, and E) or Tag/$Ghr^{-/-}$ (B, D, and E) mice were evaluated for p63 (A and B, basal cell specific), CK18 (C and D, luminal cells), or mDLP proteins (E and F). *Insets* represent negative controls for immunostaining.

mice. Testosterone levels were not affected by either Tag (Tag/$Ghr^{+/+}$ *vs.* $Ghr^{+/+}$) or GHR ($Ghr^{+/+}$ *vs.* $Ghr^{-/-}$) expression status as determined by ANOVA (Fig. 4). Furthermore, immunohistochemical analysis of AR demonstrated that there was no difference in AR expression between Tag/$Ghr^{+/+}$ and Tag/$Ghr^{-/-}$ mice in normal or cancerous prostate epithelial cells (Fig. 5).

Analysis of markers of prostate epithelial cell differentiation

To study the effect of GH signaling on prostate development and differentiation, several biomarkers were evaluated by immunohistochemistry. Markers of prostatic epithelial cell differentiation included p63 for basal cells and CK18 for the luminal cell subpopulation. Functional differentiation was assessed by immunostaining for the mDLP proteins. In Tag/$Ghr^{+/+}$ prostates, basal cells (p63+) were intermittently localized along the basement membrane in the central and distal regions of the ventral and dorsolateral lobes (Fig. 6A), and this pattern was not affected by the loss of GH signaling in the Tag/$Ghr^{-/-}$ prostates (Fig. 6B). The majority of the prostatic epithelium in both Tag/$Ghr^{+/+}$ and Tag/$Ghr^{-/-}$ mice stained for CK18, a marker of a differentiated luminal cell (Fig 6, C and D). Furthermore, in both genotypes, mDLP strongly stained in the dorsolateral prostate (Fig. 6, E and F), indicating that functional differentiation of the epithelial cells was not compromised by the loss of GHR.

Cell proliferation and apoptosis

In the C3(1)/Tag mouse, which is one of the parental strains used to generate the current Tag/*Ghr* model, Shibata *et al.* (29) reported that the severity of prostate preneoplasia correlated with proliferation and apoptosis of prostate epithelial cells. Due to the low incidence and area of PIN in Tag/$Ghr^{-/-}$ mice, we compared proliferation and apoptosis in normal-appearing prostate epithelial cells, which has potential to develop into PIN, using PCNA and the TUNEL assay, respectively. In normal-appearing prostate epithelium, proliferation was significantly decreased (Fig. 7) and apoptosis significantly increased (Fig. 8) in Tag/$Ghr^{-/-}$ mice, compared with Tag/$Ghr^{+/+}$.

Discussion

PIN lesions are thought to be precursors to prostate cancer in both man and rodents (35, 37). Morphologically, high-grade PIN and prostate cancer share spatial distribution and cytological characteristics. The transition between high-grade PIN and areas of prostatic adenocarcinoma suggest a progression of prostatic neoplasia from a noninvasive into an invasive form, with high-grade PIN representing the non-invasive phase (38).

Shibata and colleagues (29) have shown that progression of PIN to invasive prostate carcinoma in the C3(1)/Tag

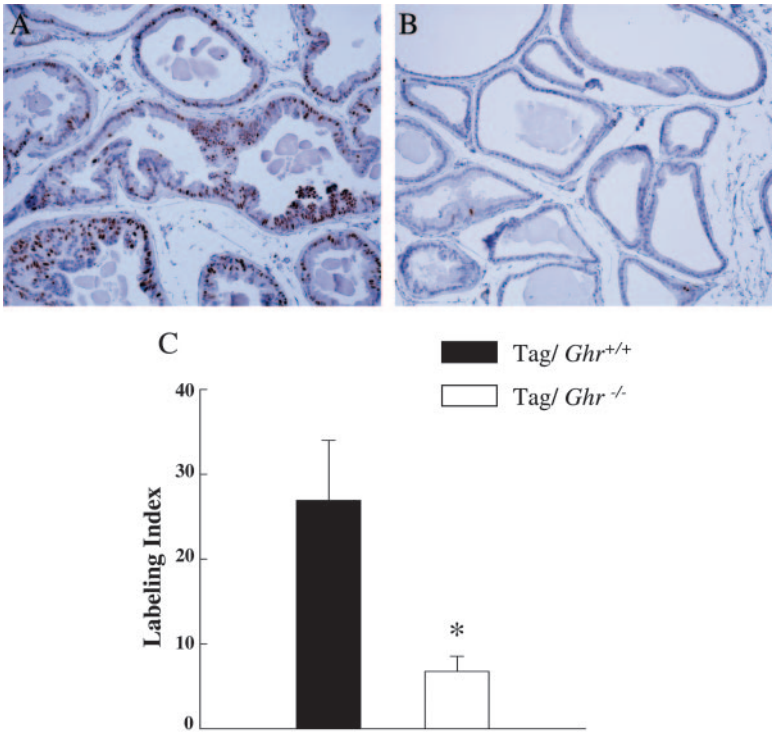

FIG. 7. Prostate epithelial cell proliferation was significantly decreased in Tag/Ghr$^{-/-}$ mice. Immunohistochemical analysis of normal-appearing ventral prostate epithelium revealed a significant reduction in PCNA expression in Tag/*Ghr*$^{-/-}$ mice (B), compared with Tag/*Ghr*$^{+/+}$ mice (A) (n = 5 per group). *, Significantly different from Tag/*Ghr*$^{+/+}$ control ($P < 0.05$; Student's *t* test.

mouse is similar to that observed in man. Our present study suggests that Tag/*Ghr*$^{-/-}$ mice, in which GH signaling has been disrupted, are resistant to prostate carcinogenesis. The Tag/*Ghr*$^{-/-}$ mouse developed PIN at a lower incidence and longer latency than the parental C3(1)/Tag strain. The Tag/*Ghr*$^{+/+}$ animals did not develop any prostate cancers by 9 months of age, whereas the C3(1)/Tag mouse has been reported to develop prostate cancers by 7 months (31). These differences are likely caused by genetic factors introduced by mating the C3(1)/Tag mouse, which is an FVB/N background, with the GHR knockout mouse, which is derived from BALB/c. Others have reported that genetic background can affect the penetrance of the C3(1)/Tag construct (39, 40). This was not a confounding issue in the current study because all mice used were derived identically (*i.e.* by crossing the C3(1)/Tag mouse with the Laron mouse). Nevertheless, the new model presented here demonstrates that the loss of GHR produced a significant reduction in the level of PIN in the ventral as well dorsal-lateral lobes in terms of incidence and PIN area.

Tag expression was evaluated by immunohistochemical analysis. As described by Shibata *et al.* (29), expression was detected at very low levels in normal epithelial cells of the prostate but increased in low-grade PIN and high-grade PIN in the Tag/*Ghr*$^{+/+}$ mice. A similar pattern of Tag expression is seen in the Tag/*Ghr*$^{-/-}$ mice. Robertson *et al.* (40) reported that Tag expression was insensitive to prolactin signaling. Here we found that GH signaling was not essential for Tag expression controlled under C3 promoter fragment. Even though fewer lesions are observed in Tag/*Ghr*$^{-/-}$ mice than Tag/*Ghr*$^{+/+}$ mice, the two genotypes had parallel Tag expression levels within each of the various degrees of PIN severity.

As noted above, it is well established that GH is important for prostate growth in full-grown, adult humans (2, 4, 41). Acromegalics have enlarged prostates that shrink to normal size in response to treatments that lower GH serum levels. Furthermore, the prostate shrinks to below normal volume in acromegalics rendered GH deficient due to aggressive therapy (2). Data from the current study suggest that GH is also important for prostate growth in the mouse. Disruption of the GHR gene in Tag/*Ghr*$^{-/-}$ mice resulted in a 60% decrease in prostate weight relative to their Tag/*Ghr*$^{+/+}$ siblings, and the decrease in prostate weight was proportional to the reduction in overall body weight, consistent with a proportional effect of disrupted GH action on prostate and body weight. Importantly, loss of GH signaling did not appear to affect epithelial cell cyto- or functional differentiation as revealed by similar expression levels and pattern of p63, CK18, and mDLP in the Tag/*Ghr*$^{+/+}$ and Tag/*Ghr*$^{-/-}$ mice. Thus, changes in carcinogenesis between the two genotypes are not likely to be a function of altered epithelial cell differentiation.

Androgens play a critical role in prostate growth, development, and carcinogenesis, and the androgen pathway has been the target of first-line prostate therapies for many years. We asked whether disrupting GH signaling resulted in a down-regulation of androgens or the expression of the AR, which could explain the lack of carcinogenesis in the Tag/*Ghr*$^{-/-}$ mice. However, as shown in Fig. 4, serum testosterone levels were affected by neither the presence of Tag (Tag/*Ghr*$^{+/+}$ *vs.* *Ghr*$^{+/+}$) nor disruption of the GHR (*Ghr*$^{+/+}$ *vs.* *Ghr*$^{-/-}$). Furthermore, AR expression was not compromised in the prostate epithelium of Ghr$^{-/-}$ mice relative to controls (Fig. 5). Therefore, we concluded that the protective effect afforded by disrupting GH signaling is independent of either serum testosterone or AR expression. This is of significance clinically because prostate cancers that initially respond to

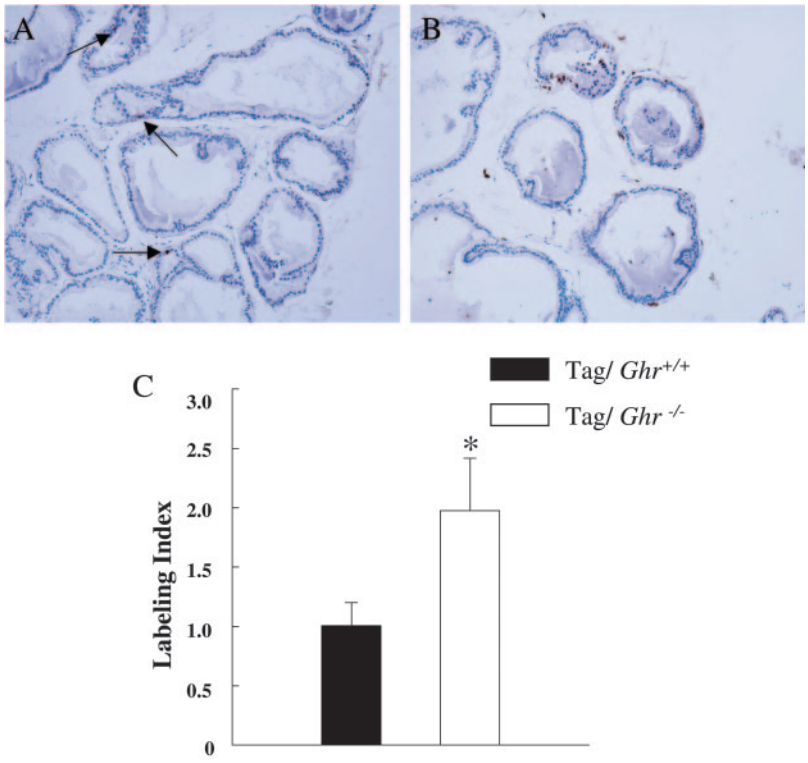

FIG. 8. Prostate epithelial cell apoptosis was significantly increased in Tag/$Ghr^{-/-}$ mice. Apoptosis was evaluated in normal-appearing ventral prostate epithelium using the TUNEL assay (n = 5 per group). A significant increase in apoptosis was observed in Tag/$Ghr^{-/-}$ mice (B), compared with Tag/$Ghr^{+/+}$ mice (A) (*lower panel*). *, Significantly different from Tag/$Ghr^{+/+}$ control ($P < 0.05$; one-tailed Student's t test).

antiandrogen therapies often evolve into androgen-independent disease, which is currently incurable.

Recently Ormandy and colleagues (40) crossed the C3(1)/Tag mouse used in the preset studies with the prolactin receptor knockout mouse ($Prlr^{-/-}$) and evaluated prostate carcinogenesis at 50 wk of age. Whereas there was no difference in PIN area in the dorsal prostate lobes, PIN area in the ventral prostate were significantly reduced in $Prlr^{-/-}$ mice relative to control mice. Furthermore, whereas one of 11 $Prlr^{+/+}$ mice and four of 21 $Prlr^{+/-}$ mice harbored prostate tumors, no prostate tumors were observed in any of the $Prlr^{-/-}$ mice (40). These data indicate that disruption of PRL signaling can impede mouse prostate carcinogenesis. However, PRL levels in the $Ghr^{-/-}$ mouse are not reduced (34), suggesting that disruption of PRL signaling is not responsible for protection from PIN development in Tag/$Ghr^{-/-}$ mice.

Carcinogenesis is characterized by dysregulated cell proliferation or apoptosis. The GH/IGF axis plays an important role in regulating prostate epithelial cell proliferation and apoptosis both *in vitro* and *in vivo* (42). One of the parental strains of the Tag/*Ghr* mouse presented in this communication is the Laron mouse, which, in addition to lacking a functional GHR, also has only about 10% of the serum IGF-I present in wild-type mice (33). We therefore hypothesized that the prostate epithelial cells of Tag/$Ghr^{+/+}$ mice would have a significant proliferation advantage, compared with Tag/$Ghr^{-/-}$ mice, resulting in more rapid progression of carcinogenesis. Our results indicate that proliferation is significantly lower and apoptosis is significantly higher in the prostate epithelium of Tag/$Ghr^{-/-}$ mice, compared with Tag/$Ghr^{+/+}$ mice (Figs. 7 and 8). Because all the prostate cells of both groups of mice harbor the same oncogene (Tag), the observed difference in prostate cell proliferation and apoptosis is likely to have a significant impact on prostate carcinogenesis.

In summary, we have crossed the C3(1)/Tag mouse with the GHR/binding protein knockout (Laron) mouse, resulting in a model in which prostate cancer progression can be assessed in the presence or absence of GH signaling. The data indicate that progression of Tag-initiated prostate epithelium is significantly inhibited in the absence of GH signaling. This inhibition is not due to insufficient Tag expression or androgen signaling in Tag/$Ghr^{-/-}$ mice relative to Tag/$Ghr^{+/+}$ mice. Rather, cancer inhibition appears to be associated with decreased proliferation and increased apoptosis of the prostate epithelium of Tag/$Ghr^{-/-}$ mice. These findings may have important translational implications. It is generally accepted that PINs are precursors to lethal prostate cancers, and these lesions occur at a similar incidence in individuals of populations at either high or low risk for the development of prostate cancer. Thus, the difference in mortality rates between high- and low-risk populations seems to be due to differences in the progression of PIN to prostate cancers. The findings presented here suggest that PIN lesions may require GH signaling for progression, suggesting that the GH signaling pathway or the GH/IGF axis may represent important targets for the development of agents to prevent prostate cancer.

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